

IT IS CLAIMED:

1. A method of detecting the presence or absence of a plurality of selected target sequences in a target polynucleotide, comprising

5 adding to a target polynucleotide, a plurality of different-sequence probe pairs, where each probe pair includes two polynucleotide probe elements which are complementary in sequence to adjacent portions of a selected one of the target sequences in the target polynucleotide, one of the elements in a probe pair contains a nonpolynucleotide polymer chain which imparts a distinctive electrophoretic mobility in a sieving matrix, to the associated probe pair, when the elements in the pair are ligated, and the other, 10 second element in the pair contains a detectable reporter label,

15 hybridizing the probe pairs with the target polynucleotide,

20 treating the hybridized polynucleotides under conditions effective to ligate the end subunits of target-bound probe elements when their end subunits are base-paired with adjacent target bases,

25 releasing the ligated probe pairs from the target polynucleotide, and

separating the released, ligated probe pairs by 30 electrophoresis in such a sieving matrix.

2. The method of claim 1, wherein the polynucleotide portions of all of the probe pairs, in ligated form, are substantially the same in length.

3. The method of claim 1, wherein prior to said releasing, said ligated probe pairs are amplified by repeated cycles of probe binding and ligation.

4. The method of claim 1, wherein the second probe element in each probe includes two alternative-sequence oligonucleotides which (i) are complementary to alternative sequences in the same portion of an associated target sequence and (ii) are derivatized with different detectable reporters, and said detecting includes determining the mutation state of each said target sequence according to (a) a signature electrophoretic mobility of each probe, which identifies the target sequence associated with that probe, and (b) a signature reporter label, which identifies the mutation state of that target sequence.

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5. The method of claim 1, wherein the first probe element in each probe includes two alternative-sequence oligonucleotides which (i) are complementary to alternative sequences in the same portion of an associated target sequence and (ii) are derivatized with different nonpolynucleotide polymer chains which impart a distinctive electrophoretic mobility to each associated probe pair, when the elements in the pair are ligated, and said detecting includes determining the mutation state of each said target sequence according to (a) a signature reporter label which identifies the target sequence associated with that probe, and (b) a signature electrophoretic mobility, which identifies the mutation state of that target sequence.

6. The method of claim 1, wherein said nonpolynucleotide polymer is selected from the group consisting of polyethylene oxide, polyglycolic acid, polylactic acid, polypeptide, oligosaccharide, and polyurethane, polyamide, polysulfonamide,

polysulfoxide, polyphosphonate, and block copolymers thereof, including polymers composed of units of multiple subunits linked by charged or uncharged linking groups.

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7. The method of claim 1, wherein said hybridizing is carried out with the target polynucleotide immobilized on a solid support; and prior to said releasing, the immobilized target polynucleotide is washed to remove probe pairs not bound to the target polynucleotide in a sequence-specific manner.

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8. A method of detecting the presence or absence of a plurality of selected target sequences in a target polynucleotide, comprising

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adding to a target polynucleotide, a plurality of different-sequence probe pairs, where each probe pair includes two polynucleotide probe elements which are complementary in sequence to adjacent portions of a selected one of the target sequences in the target polynucleotide, one of the elements in a probe pair contains a nonpolynucleotide polymer chain which imparts a distinctive elution characteristic in a chromatographic separation medium to the associated probe pair, when the elements in the pair are ligated, and the other, second element in the pair contains a detectable reporter label,

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hybridizing the probe pairs with the target polynucleotide,

treating the hybridized polynucleotides under conditions effective to ligate the end subunits of target-bound probe elements when their end subunits are base-paired with adjacent target bases,

releasing the ligated probe pairs from the target polynucleotide, and separating the released, ligated probe pairs by chromatography in such a chromatographic medium.

5 9. The method of claim 8, wherein the polynucleotide portions of all of the probe pairs, in ligated form, are substantially the same in length.

10 10. The method of claim 8, wherein prior to said releasing, said ligated probe pairs are amplified by repeated cycles of probe binding and ligation.

15 11. The method of claim 8, wherein the second probe element in each probe includes two alternative-sequence oligonucleotides which (i) are complementary to alternative sequences in the same portion of an associated target sequence and (ii) are derivatized with different detectable reporters, and said detecting includes determining the mutation state of each said target sequence according to (a) a signature elution characteristic of each probe, which identifies the target sequence associated with that probe, and (b) a signature reporter label, which identifies the mutation state of that target sequence.

20 12. The method of claim 8, wherein the first probe element in each probe includes two alternative-sequence oligonucleotides which (i) are complementary to alternative sequences in the same portion of an associated target sequence and (ii) are derivatized with different nonpolynucleotide polymer chains which impart a distinctive electrophoretic mobility to each

associated probe pair, when the elements in the pair are ligated, and said detecting includes determining the mutation state of each said target sequence according to (a) a signature reporter label of each probe, which identifies the target sequence associated with that probe, and (b) a signature elution characteristic, which identifies the mutation state of that target sequence.

10 13. The method of claim 8, wherein said nonpolynucleotide polymer is selected from the group consisting of polyethylene oxide, polyglycolic acid, polylactic acid, polypeptide, oligosaccharide, and polyurethane, polyamide, polysulfonamide, polysulfoxide, polyphosphonate, and block copolymers thereof, including polymers composed of units of multiple subunits linked by charged or uncharged linking groups.

20 14. The method of claim 8, wherein said hybridizing is carried out with the target polynucleotide immobilized on a solid support; and prior to said releasing, the immobilized target polynucleotide is washed to remove probe pairs not bound to the target polynucleotide in a sequence-specific manner.

25 15. A method of distinguishing different-sequence polynucleotides electrophoretically in a sieving medium, comprising forming one or more different-sequence polynucleotide(s), each different-sequence polynucleotide containing (i) a detectable reporter label and (ii) an attached polymer chain which imparts to each different-sequence polynucleotide, a

distinctive electrophoretic mobility in a sieving matrix,

fractionating said polynucleotide(s) by capillary electrophoresis in a sieving matrix, and detecting the fractionated polynucleotide(s).

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16. The method of claim 15, wherein the polymer chains have substantially the same lengths, and said different-sequence polynucleotide(s) have different lengths.

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17. The method of claim 16, for sequencing DNA by dideoxy chain termination, wherein said polynucleotides are formed using a 5'-primer to which said polymer chain is covalently bound.

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18. The method of claim 17, wherein said different-sequence polynucleotides terminate at their 3'-ends with dideoxynucleotides that are covalently labeled with spectrally resolvable dyes effective to distinguish the 3'-terminal nucleotide of each different-sequence polynucleotide.

19. The method of claim 15, for detecting one or more target sequences in a sample, wherein said forming includes the steps of

25 providing one or more different-sequence probe(s), each different-sequence probe containing (i) a detectable reporter label and (ii) an attached polymer chain which imparts to each different-sequence polynucleotide, a distinctive electrophoretic mobility in a sieving matrix,

30 reacting said probe(s) with a target polynucleotide under conditions effective to allow 35 hybridization of sequence-complementary probe(s) with

the target polynucleotide, where the target polynucleotide is immobilized on a solid support before or after said reacting,

5 washing the immobilized target polynucleotide to remove probe(s) not bound to the target polynucleotide in a sequence-specific manner, and

10 denaturing the target polynucleotide to release different-sequence probe(s) hybridized to the target polynucleotide, to form different-sequence polynucleotides for electrophoretic separation.

20. The method of claim 15, wherein said different-sequence probe(s) have substantially the same length.

15 21. The method of claim 20, for detecting one or more target sequences in a sample, wherein said forming includes the steps of (i) adding to a target polynucleotide one or more sequence-specific probes, each probe having first and second probe oligonucleotides which are effective to bind to adjacent regions in a target sequence, and where one of said first and second probe oligonucleotides is derivatized with said polymer chain, (ii) reacting the probe(s) with the target polynucleotide under conditions effective to bind said first and second probe oligonucleotides in each probe to their specific target sequences, (iii) ligating said oligonucleotides bound to the target polynucleotide under conditions effective to ligate the end subunits of target-bound oligonucleotides when their end subunits are base-paired with adjacent target bases, to form ligated probe(s), and (iv) releasing the ligated probe(s) from the target polynucleotide to form said different-sequence polynucleotide(s).

22. The method of claim 21, wherein said polymer chain is attached covalently to said first probe oligonucleotide, and each said second probe oligonucleotide is reporter-labeled.

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23. The method of claim 21, wherein prior to said releasing, said ligated probe(s) are amplified by repeated cycles of probe binding and ligation.

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24. The method of claim 21, wherein the second probe oligonucleotide in each probe includes two alternative-sequence oligonucleotides which (i) are complementary to alternative sequences in the same portion of an associated target sequence and (ii) are derivatized with different detectable reporters, and said detecting includes determining the mutation state of each said target sequence according to (a) a signature electrophoretic migration rate of each probe, which identifies the target sequence associated with that probe, and (b) a signature reporter moiety, which identifies the mutation state of that target sequence.

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25. The method of claim 15, for detecting one or more target sequences in a sample, wherein said forming includes the steps of (i) adding to a target polynucleotide one or more sequence-specific probes, each probe having first and second sequence-specific primer oligonucleotides which are effective to hybridize with opposite end regions of complementary strands of a target polynucleotide segment, respectively, where the first primer oligonucleotide in each probe is derivatized with a probe-specific polymer chain, (ii) reacting the probe(s) with the target polynucleotide under conditions effective to

bind both primer oligonucleotides to opposite end regions on complementary strands of the target polynucleotide, and (iii) amplifying the target segment(s) by primer-initiated polymerase chain reaction to form amplified different-sequence polynucleotide(s).

26. The method of claim 25, wherein each said second primer oligonucleotide is reporter-labeled, and said different-sequence polynucleotide(s) are double stranded.

27. The method of claim 15, wherein said forming includes the steps of (i) adding to a target polynucleotide one or more sequence-specific probes, each probe containing a first single-stranded DNA segment, and a second segment which includes single-stranded RNA, wherein the polymer chain is attached to said first segment, and the detectable reporter label is attached to said second segment, (ii) reacting the probe(s) with the target polynucleotide under conditions effective to bind said first and second segments to their specific target sequences in the target polynucleotide, (iii) reacting hybridized probe with an RNase enzyme specific for RNA/DNA substrate, to form modified, labeled probe(s) lacking the polymer chain, and (iv) releasing both unmodified and modified, labeled probe(s) from the target polynucleotide to form said different-sequence polynucleotide(s).

28. A probe composition for use in detecting one or more of a plurality of different sequences in a target polynucleotide, comprising

5 a plurality of sequence-specific probes, each characterized by (a) a binding polymer having a probe-specific sequence of subunits designed for base-specific binding of the polymer to one of the target sequences, under selected binding conditions, and (b) attached to the binding polymer, a polymer chain which imparts to each probe, a distinctive electrophoretic mobility in a sieving matrix.

10 29. The composition of claim 28, wherein said polymer chain is selected from the group consisting of polyethylene oxide, polyglycolic acid, polylactic acid, polypeptide, oligosaccharide, and polyurethane, polyamide, polysulfonamide, polysulfoxide, and block copolymers thereof, including polymers composed of units of multiple subunits linked by charged or uncharged linking groups.

20 30. The composition of claim 28, wherein each sequence specific probe further includes a second binding polymer having a reporter, where the first-mentioned and second binding polymers in a sequence-specific probe are effective to bind in a base-specific manner to adjacent and contiguous regions of a selected target sequence, allowing ligation of the two binding polymers when bound to the target sequence in a sequence-specific manner, and the polymer chain attached to the first binding polymer imparts to each ligated probe pair, a distinctive electrophoretic mobility in a sieving matrix.

25 31. The composition of claim 28, wherein each sequence specific probe further includes a second binding polymer, where the first-mentioned and second

binding polymers in a sequence-specific probe are effective to bind in a base-specific manner to opposite end regions of opposite strands of a selected duplex target sequence, allowing primer initiated polymerization of the target region in each strand, and the polymer chain attached to the first binding polymer imparts to each polymerized region, a distinctive electrophoretic mobility in a sieving matrix.

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32. The composition of claim 28, wherein each sequence-specific probe includes a binding polymer composed of a first single-stranded DNA segment and a second segment which includes single-stranded RNA, wherein a polymer chain is attached to said first segment; a detectable reporter is attached to said second segment, and each polymer chain imparts to the probe to which said polymer chain is attached, a distinctive electrophoretic mobility in a sieving matrix.

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